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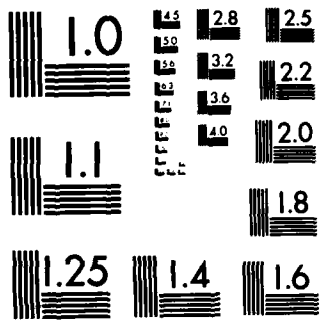

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Work thus far has focused on a class of tricylic antidepressants (TADS) whose action has a gradual onset associated with the metabolic response to the drugs. Chronic treatment of rats with TADS sensitized synaptosomal adenylate cyclase (AC) to activation with guanyl nucleotides. The research has attempted to describe anatomical and functional localization of the response, probe the biochemical mechanism of observed effects; and look for behavioral correlates of biochemical changes. An interesting finding is that unlike the hypothalamic or cortical enzyme, enzyme, cerebellar synaptosomal AC is not sensitized to

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to nucleotide activation. Other TAD associated changes are seen in cerebellum. A number of plausible biochemical mechanisms for the observed TAD responses have been investigated, and several additional possibilities have been identified. The precise mechanisms involved remains unclear. Several behavioral tests were employed to attempt to quantify observed behavioral differences between control and TAD-treated animals. The most significant observed differences was in the range and variance of responses between groups. More sophisticated and sensitive experimental paradigms are being developed.

AFOSR-TR- 84-0719

# PROGRESS REPORT

# PERFORMANCE ENHANCEMENT

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
AFOSR-MIPR-82-00064

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Progress Report: Abstract
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The general objective of this work is the enhancement of human performance without undesirable sequellae. Work thus far has focused on a class of psychoactive agents, the tricyclic antidepressants (TADs), whose action has a gradual onset associated with the metabolic response to the drugs. The principal investigator's research group has previously demonstrated that chronic treatment of rats with TADs sensitized synaptosomal adenylate cyclase (AC) to activation with guanyl nucleotides. We have attempted to extend this basic finding in three ways:

a) describe anatomical and functional localization of the response
b) probe the biochemical mechanism of observed effects
c) look for behavioral correlates of biochemical changes.

We have found that unlike the hypothalamic or cortical enzyme, cerebellar synaptosomal AC is not sensitized to guanyl nucleotide activation. Other TAD associated changes are seen in cerebellum.

A number of plausible biochemical mechanisms for the observed TAD responses have been investigated, and several additional possibilities have been identified. The precise mechanism involved remains unclear, however.

Several behavioral tests were employed to attempt to quantify observed behavioral differences between control and TAD-treated animals. The most significant observed difference was in the range and variance of responses between groups; more sophisticated and sensitive experimental paradigms are being developed.

Significant progress has been made in the development of our advanced system for magnetoencephalography. This system will allow noninvasive monitoring of evoked or spontaneous neural activity, and permit more rigorous systemic evaluation of intervention strategies.

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH (AFSC)

Chief, Technical Information Division

PROGRESS REPORT
AFOSR-MIPR-82-00064
PERFORMANCE ENHANCEMENT

## Introduction

The general objective of this work is the enhancement of human performance - to develop the capacity to elicit, prolong or transcend normal peak performance without undesirable sequellae. While a variety of putative modulators of performance and strategies for intervention and assessment have been identified, our work thus far has focused on a particular class of psychoactive agents, the tricyclic antidepressants (TADs). Unlike most pschoactive pharmaceuticals, TAD action has a gradual onset followed by a stable plateau of effectiveness. The metabolic response to TADs appears associated with (rather than destructive of) their intended effects. For this reason, these agents are useful prototypes for sustained pharmacological modulation of performance. A better understanding of the metabolic response and of the correlation between kinetics of biochemical and behavioral changes are therefore of great interest.

Previous work by the principal investigator's research group at Yale on the possible biochemical basis of TAD action is described in the attached reprint from SCIENCE (Appendix I). The work demonstrates that chronic treatment of rats with tricyclic antidepressants sensitizes the adenylate cyclase of synaptosomal membranes to activation with guanyl nucleotides. We have attempted to extend and further characterize this basic finding in three ways:

a. Describe any anatomical or functional localization of the response.
b. Probe the biochemical mechanism of the observed effects.
c. Look for behavioral correlates of the biochemical changes.

## A. Anatomical Localization

### Regional or Cellular Specialization

In synaptosomal preparations from our first series of experimental animals we have reproduced and extended the results reported from the Yale studies; i.e. synaptomal adenylate cyclase from cortex and hypothalamus of TAD- treated animals was activated by the GTP analog Gpp(NH)p to about twice the extent of synaptosomal cyclase from controls. In a new series of experiments we have found, for the first time, that synaptosomes prepared from cerebellum did not exhibit this striking effect. This data suggests a previously undefined neuroatomical or cellular specialization for the molecular effects of tricyclic antidepressants (Table I).

This observation is complicated by yet another new finding. A different biochemical probe of a mammalian synaptosomal cyclase system (see below) reveals TAD dependent effects in the cerebellum as well as other brain regions.

B. Biochemical Mechanism

In our earlier proposal we identified a number of possible biochemical loci which could, in principle, serve as loci for the observed changes with tricyclic antidepressant treatment. These include:

1. Enhanced association between the GTP binding protein activator (G) of cyclase with the catalytic moiety.
2. Increased numbers of G units.
3. Changes in the reaction of kinetics or affinity of the binding of GTP to G.
4. Enhanced communication between agonist or antagonist receptor and the G unit.
5. Decreased function or level of a putative inhibitor of adenylate cyclase.
6. G/GTP mediated inhibition of the cAMP catabolic enzyme, phosphodiesterase (PDE).

Data emerging from this and other laboratories over the past year have suggested several additional possibilities. These include:

7. Changes in function or reduction in level of an inhibitory GTP binding protein.
8. Changes in the interaction or function of the component subunits of the GTP binding protein complex.
9. Changes in function or level of one or several auxiliary proteins involved in the activation of G.

1. Enhanced Association

Since G and the cyclase catalytic moiety are both membrane associated proteins, enhanced interaction might result from changes in membrane fluidity. We examined the kinetics of relaxation of flourescence anisotorpy using hydrophobic fluorescent probes excited by polarized light. We did not observe significant differences between the membranes of TAD-treated and control animals.

Our studies of G unit regulation of photoreceptor PDE indicate that G activates this enzyme by physically interacting with an inhibitory subunit. G and inhibitor are subsequently released from the membrane, and can be measured in the supernatant following centrifugation. Kinetics of G release and return to the membrane may be resolved readily at ice temperature. We are attempting to use a similar experimental strategy to probe the interaction of G with the cyclase complex.

2. Quantitation of G

Our previous work at Yale demonstrated that a subset of synaptosomal G units can be solubilized with colchicine in isotonic buffers (Appendix II). In preliminary experiments we attempted to measure colchicine solubilized G using gamma aminonapthalene sulphanyl GTP, a flourescent analog of GTP. Although this probe has proven extremely useful for studies of a related GTP

binding protein discovered by our group and others in retinal photoreceptors, we have not achieved the requisite sensitivity to measure soluble cyclase G. We are investigating strategies to preload G while eliminating excess probe, as well as exploring alternative strategies. Measurement of radiolabeled Gpp(NH)p binding to membranes by filtration assay also appears to provide a useful approach.

3. Changes in GTP Binding to G

We have used ANS GTP to study the kinetics of binding of GTP to the G unit of photoreceptors. We have also demonstrated that bound probe may be removed by competition with unlabeled guanyl nucleotides or analogs, allowing us to quantify binding affinities. Our efforts to apply these techniques to the cyclase system thus far have been hampered by signal to noise considerations. Our effort to preload G and remove free ANS GTP should, if successful, permit such measurements to be made.

4. Enhanced Receptor/G Communication

This did not appear to be a likely basis for our observed effects since exogenous agonist was not required for cyclase activation by Gpp(NH)p. This does not obviate the possibility since tightly bound endogenous agonists might be present. Other laboratories are actively studying the modulation of agonist binding by guanyl nucleotides following chronic TAD or control treatments.

5. Changes in Cyclase Inhibitor

The existence of a cyclase inhibitor has not yet been proven, although our work has lent the idea greater credibility. We have isolated and further characterized the inhibitor of photoreceptor PDE. We have demonstrated that this heat-stable protein complex also inhibits the adenylate cyclase of brain and other tissue.

6. Inhibition of PDE

We have performed a number of control experiments and have found no evidence of a TAD sensitive phosphodiesterase in brain tissues. In current cyclase studies we routinely employ both internal and external controls for phosphodiesterase activity.

7. Inhibitory G Units

Recent studies of adenylate cylclase have underscored the role of inhibitory GTP binding proteins in the modulation of the cyclase catalytic moeity. A deficit in the TAD treated inhibitory G system might account for the observed biochemical changes. The effect of an adenosine analog N6, R-phenyl isopropyl adenosine (PIA) on synaptosomal cyclase activity was studied since adenosine has been suggested to act through the inhibitory G system. However, PIA is far more effective as a cyclase inhibitor in brain tissue from TAD treated animals than controls (Table II). This suggests that TADs may produce region-specific enhancements of both stimulatory and inhibitory G units; alternatively PIA interacts with other molecular aspects of these transmembrane switching devices.

8. Changes in G Subunits

For a number of years we have realized that G activator proteins are complexes of several subunits and that the coordinated interactions of these components is critical for the proper control of cyclic nucleotide metabolism. While one subunit was shown to actually bind GTP, the specific function of the others was not understood.

This year we have succeeded in physically and functionally resolving the subunits of the photoreceptor G. One subunit catalyzes hydrolysis of bound GTP while another permits binding to occur. These techniques will eventually permit us to resolve the locus of G unit changes if such changes induced by the tricyclic anticepressants.

We have continued to develop a pragmatic experimental basis for treating the photoreceptor G as a model system for the cyclase. In component exchange experiments we demonstrated that photoreceptor G can activate cyclase as can the photoreceptor signal protein, rhodopsin. In a collaboration with Lutz Birnbaumer of Baylor, we are examining 2 dimension peptide maps of the phosphodiesterase G with those of the brain cyclase G systems.

In our second series of experimental animals we are attempting to isolate photoreceptors to compare the transduction proteins in TAD and control animals. Initial problems with these experiments indicate that we need more efficient procedures to harvest the smaller rat rods. The photoreceptor system remains a particularly attractive model because of the abundance of G and the relative ease of obtaining it.

We are developing antibodies to purified preparations of G protein and other components from the photoreceptor system. These will be tested for cross reactivity expected with cyclase G. Availability of antibodies will significantly enhance our ability to dissect the system.

9. Auxiliary proteins

In the photoreceptor system we have discovered several peripheral or soluble proteins which participate in GTP-dependent regulation of cyclic nucleotide metabolism. We have identified a new protein where catalyzes the release of bound GDP so that GTP can be subsequently bound and the complex activated. We also have preliminary evidence that such a function is found in the cyclase inhibitory G system, and that it may in fact be the rate limiting step in G activation.

10. Measurement of Cyclic Nucleotide Levels

While the enzymological effects we have observed are significant and interesting, they are most likely to affect cellular physiology if they are reflected in the metabolic profile of the cell. For this reason we have developed the technical capability and experimental paradigms to monitor levels of the various nucleotides in rapidly frozen tissue samples.

We have now outfitted our HPLC for gradient separation of the nucleotide phosphates using a fully charged strong anion exchange column. We have

developed gradients and protocols optimized for rapid or high resolution nucleotide determinations. Our system is now suitable for preparative, as well as analytical, tasks so that stock nucleotides may be purified for use in sensitive allosteric roles. We have set up the system for repetitive, unattended operation such as required for enzymological application. We also have begun to experiment with using another column for resolution of G system proteins.

We have tested two techniques for extraction of nucleotides from tissue samples. Each approach involves initial extraction of the crushed, frozen tissue sample with a mixture of perchloric acid and ethanol. The extract is then neutralized with analiquot of KOH. In a modified second approach the perchlorate solution is extracted with fluorocarbon. This procedure is more tedious but avoids precipitation.

## C. Behavioral Studies

### TAD Responses

During the 3 weeks of injections of the second series of experimental animals, we noticed significant behavioral differences in the TAD treated animals. Treated animals appeared to become hypersensitive and hyperkinetic in response to the daily injections. They also became more prone to aggressive behavior among themselves and directed toward the handler.

Before sacrificing the animals we ran some simple behavioral tests in an attempt to quantify our observations. One test examined exploratory behavior. The rat was placed in a box in the center of a darkened empty room. The floor had been divided in a grid with labeled squares. The box was lifted remotely and records of the time sequence and number of blocks entered by the rat were kept by two observers. A light avoidance trial was conducted similarly except that a second box with entry hole was placed in a corner of the room which was brightly lighted. We measured the time required for the rat to enter the haven box after the starting box was lifted.

Mean exploration pathlengths and times for light avoidance were not significantly different between TAD treated and control gorups. However, there were major differences in the range and variance of trial values between groups. While control animals demonstrated fairly consistent responses, the TAD animal responses were very inconsistent. Some of the animals began moving quickly and moved constantly; others scarcely moved from the original square.

We plan a number of improvements for the next series of trials. We have obtained video equipment so that sessions may be recorded and more precisely scored. We also plan to compare TAD effects on the performance of a learned task and on evoked responses measured by magnetoencephalography.

### MEG Development

Advanced techniques for magnetoencephalographic (MEG) monitoring of neural activity should prove an important tool for studies of performance enhancement. Since MEG is noninvasive and does not require a cooperative subject, it can be used to study man or experimental animals. Intrisic

properties of magnetic fields associated with neural activity permit precise spatial localization of the nerves that produced the fields. We are developing at Los Alamos an array of sensitive miniature magnetic sensors and a system of data acquisition electronics and computer which will greatly facilitate high resolution evolved field mapping and allow localization of spontaneous activity. The systems will be used to monitor emotional and cognitive states and to look for pharmacological signatures in evoked and spontaneous neural activity. We will also attempt to identify and evaluate neural components of task performance and see how these are affected by intervention strategies.

We have made significant progress in the development of our magnetoencephalography system. We have:

1. Obtained and assembled electronics for data collection and sensory stimulation.
2. Interfaced these with our HP 9000 computer system.
3. Fabricated liquid helium dewar suspension and manipulation systems.
4. Developed experimental systems software.
5. Established private sector collaborations for development of the multisensor SQUID array.
6. Run preliminary experiments with our single channel gradiometer system.

## TABLE I

**Comparison of the effects of antidepressant treatment on Gpp(NH)p stimulation of adenylate cyclase in membranes from cerebral cortex and cerebellum.**

**Adenylate Cyclase Activity (% of Mn Stimulated Activity)**

| | Cerebral Cortex | | Cerebellum | |
|---|---|---|---|---|
| | Saline treated | DMI treated | Saline treated | DMI treated |
| [GPP(NH)p] | | | | |
| - | 26 | 26 | 20 | 19 |
| $10^{-8}$M | 27 | 31 | 17 | 20 |
| $10^{-7}$M | 57 | 74 | 34 | 35 |
| $10^{-6}$M | 72 | 92 | 58 | 62 |

Rats were injected intraperitoneally with sterile 85% saline or 10mg/Kg Desipramide HCl for 16 days. Membranes were prepared from cerebral cortex and cerebellum after decapitation as described in (Rasenick & Bitensky PNAS 77: 4628 (1980)). Adenylate cyclase was assayed as described in (Menkes, et al. Appendix II) except the ATP regenerating system used was 12 mM phosphocreatine, 100 mg/ml creatinephosphokinase. Adenylate cyclase activities are expressed as percent of the activity measured in the presence of 10mM $MnCl_2$ (a measure of maximum catalytic activity). For cerebral cortex membranes, $MnCl_2$ stimulated adenylate cyclase activity was 559$\pm$ 14 pmole/min/mg protein for both treated and untreated groups. For cerebellum membranes, $MnCl_2$ activity was 256$\pm$ 29 pmole/min/mg protein.

TABLE II

Inhibition of adenylate cyclase by $N^6$(R-Phenyl-isopropyl) -adenosine (PIA) in cerebellum membranes from DMI or saline treated rats.

| | % Inhibition | |
|---|---|---|
| | Saline treated | DMI treated |
| (PIA) | | |
| $10^{-6}$M | 19 | 55 |
| $10^{-5}$M | 56 | 98 |

Membrane adenylate cyclase was assayed as described in Table I except that membranes were pretreated for 30 minutes with adenosine deaminase 2.5U/ml to deplete endogenous adenosine, and the adenylate cyclase reaction mixture contained no IBMX, 5U/ml adenosine deaminase and 100 μ M GTP. PIA was made up in DMSO and equivalent amounts of DMSO were added to control membranes. In hypothalamus membranes $10^{-6}$M PIA inhibited adenylate cyclase in controls 15%, and in treated, 41%.

TABLE III

Investigative behavior of saline control and TAD treated rats

| | Control | DMI | IMI | AMI |
|---|---|---|---|---|
| # trials | 6 | 6 | 6 | 4 |
| # squares | | | | |
| mean | 27.5 | 14.5 | 21.0 | 33.75 |
| variance | 68.9 | 120.9 | 330.3 | 110.2 |
| # boundary crossings | | | | |
| mean | 39.3 | 23.0 | 27.8 | 49.8 |
| variance | 64.6 | 248.0 | 474.1 | 216.7 |

Rats were injected intraperitoneally with sterile saline or 10mg/Kg TAD (Desiprimine = DMI, Imiprimine = IMI, Amitriptyline = AMI) for 21 days. On the day before sacrifice, a standard test of investigative behavior was performed. After 18 hours of dark adaptation, rats were placed into a square room illuminated with dim red light. The floor was marked into a grid of 12x12 25cm squares. A starting box was lifted and 2 observers recorded the rat's movements for 2 minutes using prepared scoring sheets. The number of squares investigated and the number of crossings of square boundaries by each rat were subsequently tabulated.

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